Conventional Bladder Wash Cytology Performed by Four Experts *versus* Quantitative Image Analysis

H.G. van der Poel, M.D., Ph.D., M.E. Boon, M.D., Ph.D., P. van Stratum, E.C.M. Ooms, M.D., Ph.D., H. Wiener, M.D., Ph.D., F.M.J. Debruyne, M.D., Ph.D., J.A. Witjes, M.D., J.A. Schalken, Ph.D., W.M. Murphy, M.D., Ph.D.

Department of Urology, University Hospital, Nijmegen (HGvdP, PvS, FMJD, JAW, JAS); Leiden Cytology and Pathology Laboratory, Leiden (MEB); Department of Pathology, Westeinde Hospital, The Hague, The Netherlands (ECMO); Department of Urology, Urology, University Hospital, Vienna, Austria (HW); and Department of Pathology and Laboratory Medicine, Division of Anatomic Pathology, College of Medicine, University of Florida, Gainesville, Florida (WMM)

Bladder wash cytology provides superior results for the detection of bladder malignancies than does voided urine analysis. Image analysis systems have been developed for quantification of cytologic features. In this study, routine bladder wash cytology is compared with an automated image analysis system (QUANTICYT). We studied a random set of 100 bladder wash samples from a population of 1614 patients in follow-up after bladder cancer. Four experienced pathologists interpreted the same 100 Papanicolaou-stained slides. Cytologic and image analysis results were compared for prediction of a cystoscopic lesion, histologic abnormalities, and tumor recurrence. After application of receiver operating characteristic curves, prediction of a cystoscopic lesion by cytology and image analysis was comparable. Both the image analysis system and the cytologic examination detected all of the high-grade lesions. Image analysis was superior to cytologic analysis for the prediction of tumor recurrence after normal findings at cystoscopic examination.

KEY WORDS: Bladder cancer, Bladder washing, Cytology, Follow-up, Image analysis.

Mod Pathol 1997;10(10):976–982

Cytologic analysis is important for the assessment of mucosal changes in the bladder. With a conventional light microscope, changes related to tumor growth can be identified by experienced cytopathologists (1). The superior sensitivity of bladder wash cytology (BWC) over voided urine analysis was shown by Zein *et al.* (2). Different methods were studied to enhance the detection of bladder neoplasms in cytologic material. DNA ploidy is a sensitive method (3–7). High-grade tumors are aneuploid, whereas many intermediate-grade and almost all low-grade lesions are diploid (8) and thus can not be discriminated from normal samples on the basis of flow cytometric analysis alone. Recent studies applying image cytometric analysis for ploidy determination on bladder washings, however, showed a sensitivity of 91%, compared with 71.4% for flow cytometric analysis (4). The visual control of selected cells for analysis by image cytometric (9) techniques proved an important advantage over flow cytometric analysis.

A recent publication presented the results of a combination of ploidy and nuclear shape analysis for evaluation of bladder wash specimens for the prediction of tumor recurrence and progression (10). DNA ploidy analysis has proved to be useful for the detection of high-grade neoplasms, whereas nuclear shape abnormalities have been more useful for detecting low-grade tumors (10). The present study was conducted to compare the bladder wash quantitative cytologic image analysis system, QUANTICYT (BioProcon, Wijchen, The Netherlands), to light microscopic interpretation of BWC. Four experts in bladder cytology (MEB, WMM, ECMO, HW) interpreted the same 100 cytologic samples. Each pathologist used a cytologic scoring system most familiar to him or her. In addition, the samples were analyzed by bladder wash karyometry (BWK), using the QUANTICYT system. Cystoscopic, histologic, and follow-up data were available.

0893-3952/97/010010-0976$3.00/0 MODERN PATHOLOGY

VOL. 10, NO. 10, P. 976, 1997 Printed in the U.S.A.
Date of acceptance: August 5, 1997.
Address reprint requests to: H.G. van der Poel, M.D., Ph.D., Department of Urology, University Hospital Nijmegen, Post Office Box 9101, 6500 HB Nijmegen, The Netherlands; e-mail: h.vanderpoel@uro.azn.nl; fax: 0031–24-3541031.

MATERIALS AND METHODS

In a database of 1910 bladder wash samples present in the Department of Urology, University Hospital, Nijmegen, The Netherlands, 100 samples were chosen at random from the years 1993 and 1994. All of the samples were taken before cystoscopic examination from patients being followed for bladder cancer. At cystoscopy, a bladder wash sample was obtained by rinsing the bladder at least twice with 50 mL of saline solution (0.9% sodium chloride solution in distilled water). The material was instantly combined with a polyethylene glycol–based preservative (50% ethanol, 2% polyethylene glycol; molecular weight, 1500). After preservation for at least 24 hours, the samples were processed at room temperature in a Cytospin centrifuge (Shandon, Woburn, MA). Slides thus obtained were stained according to the Papanicolaou method for conventional light microscopic examination. A second Cytospin slide from the same sample was stained using Feulgen reagent for BWK according to a method previously described (10).

Four pathologists, expert in bladder cytology (ECMO, HW, MEB, WMM), participated in the study. Each of them reviewed all of the 100 Papanicolaou-stained slides without knowledge of the BWK results or clinical findings. The scoring systems used by the pathologists differed, but each scheme separated samples into categories of positive, negative, suspicious, and atypical cytology (Table 1). For the purpose of this study, both cytologic and BWK analysis were reduced to "tumor" or "no tumor" (Table 1). The BWK score was categorized into low, intermediate, or high grade; the latter two were considered as positive for tumor (10). The BWC and BWK scores were compared with respect to the presence of a lesion at cystoscopy, histologic findings in the tissue specimen, and follow-up.

TABLE 1. Cystoscopic Findings at Time of Bladder Wash Sample Compared with Bladder Wash Cytology for Different Pathologists and QUANTICYT System (n = 100)

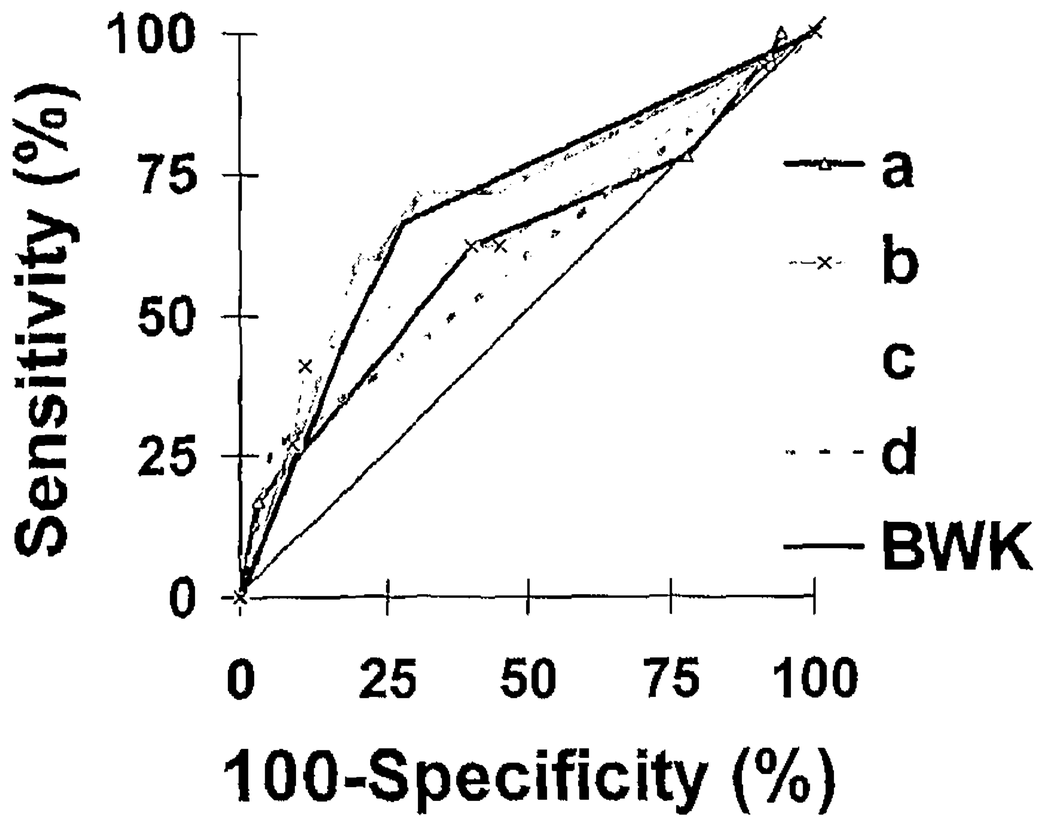
	Overall score	n	Cystoscopy lesion		No recurrence	Recurrence
			Yes	No		
Pathologist A						
No diagnosis	No tumor	4	–	4	3	1
Inflammation	No tumor	6	2	4	2	2
Other	No tumor	2	1	1	1	–
Negative	No tumor	16	5	11	10	1
Atypia	No tumor	27	4	23	16	7
Grade 1 tumor	Tumor	16	6	10	7	3
Grade 2 tumor	Tumor	23	9	14	12	2
Grade 3 tumor	Tumor	6	5	1	1	–
Total		100		68		
Pathologist B						
Negative	No tumor	48	8	40	29	11
Dysplasia	No tumor	7	–	7	7	–
Dysplasia, rule out low-grade neoplasm	Tumor	11	5	6	5	1
Dysplasia, rule out high-grade neoplasm	Tumor	1	–	1	1	–
Low-grade tumor	Tumor	15	8	7	5	2
High-grade tumor	Tumor	18	11	7	5	2
Total		100		68		
Pathologist C						
Insufficient material	No tumor	6	1	5	4	1
Inflammation	No tumor	2	–	2	1	1
No classification	No tumor	2	1	1	–	1
Negative	No tumor	46	10	36	29	7
Atypia	No tumor	22	7	15	12	3
Suspicious for tumor	Tumor	8	4	4	3	1
Low-grade tumor	Tumor	2	1	1	1	–
High-grade tumor	Tumor	12	8	4	2	2
Total		100		68		
Pathologist D						
No diagnosis	No tumor	5	–	5	4	1
Negative	No tumor	45	14	31	25	6
Low-grade tumor	Tumor	39	10	29	20	9
High-grade tumor	Tumor	11	8	3	2	1
Total		100		68		
Bladder wash karyometry						
Low risk	No tumor	64	14	50	43	7
Intermediate risk	Tumor	13	4	9	4	5
High risk	Tumor	23	14	9	5	4
Total		100		68		

Each patient underwent a cystoscopic examination after bladder wash material was obtained. A flexible or rigid cystoscope was inserted, and each malignant-looking mucosal lesion was characterized as being papillary, solid, or suspicious for carcinoma *in situ* (CIS). Histologic analysis was performed in cases in which transurethral resection of a lesion was performed. Tumor grading was according to the WHO classification; tumor staging was according the TNM classification (Ta, noninvasive papillary carcinoma with no invasion of basement membrane; T1, tumor invasion into the subepithelial connective tissue; T2, invasion into the superficial bladder muscles; and T3, invasion into the deep bladder muscle of perivesical fatty tissue). Tis or CIS is characterized by high-grade tumor cells not invading the basement membrane with a increased tendency to exfoliate into the bladder lumen. Follow-up, including cystoscopic examination of each patient, of at least 24 months was available in every case. During follow-up, histologically proved tumor recurrence or progression was documented.

For comparison of different light microscopic cytologic interpretations with the quantitative cytologic system, the area under the receiver operating characteristic (ROC) curve was used (11). In these curves, sensitivity and specificity are plotted as a function of different cut-off points of a test. The area under the curve (AUC) represents the overall value of the test. The maximal AUC is one. Recurrence-free survival intervals were evaluated with Kaplan-Meier curves and log-rank tests using SPSS/PC+ software, version 6.1 (SPSS, Chicago, IL). For multivariate evaluation of prognostic information, multivariate Cox regression analysis was used: stepwise, forward conditional. Statistical significance was assumed when P was less than .05.

RESULTS

Cystoscopic examination at the time of bladder wash sampling detected a bladder lesion in 32 cases (32%). The correlation of positive cystoscopic findings and the scoring by the different cytologists is presented in Table 1. Sensitivity for prediction of a cystoscopic lesion ranged from 58 to 63% and specificity from 63 to 91% among the four pathologists. Table 1 also presents the BWK scores. When intermediate-risk and high-risk BWK scores were judged as abnormal, the sensitivity was 56% and specificity 74% for BWK. To compare the different scoring methods applied among cytologists with the automated method, the ROC curve method was applied. In Fig. 1, the ROC curves and the areas under them for the different pathologists and the BWK are presented. The mean AUC for the four experts was 0.645 ± 0.029. The AUC for the BWK was 0.670, and



AUC (area under the ROC curve)			
BWC		AUC	s.e.
	a.	0.636	0.061
	b.	0.667	0.060
	c.	0.670	0.060
	d.	0.608	0.062
BWK (QUANTICYT)		0.670	0.060

FIGURE 1. ROC curves for the prediction of a lesion in the bladder at cystoscopic examination (n = 100). The thin, unmarked line (—) represents the AUC value of 0.5 that would be obtained when random sample assignment was applied.

the standard error was 0.060. Only one expert had an AUC value equal to that of the BWK, but none of the differences was statistically significant.

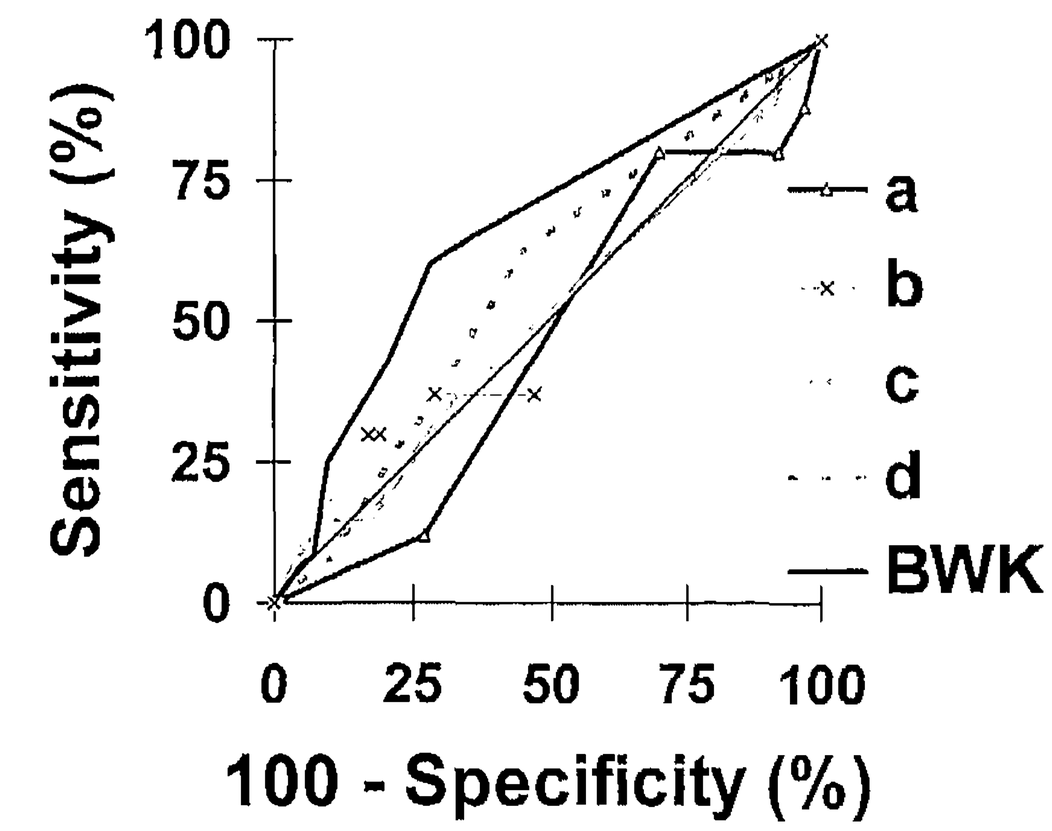
Histologic resection of a malignant lesion was performed in 18 patients within 2 months after the bladder wash sample. The following transitional cell neoplasms were found: 4 CIS, 10 pTa, 1 pT1, 3 pT2. In Table 2, the BWC and BWK scores are given. Overall sensitivity for the BWK was 13 (72%) of 18; when for each sample only the highest score of all of the four pathologists was considered, sensitivity for cytology was exactly the same. For the individual pathologists, sensitivity was 67, 56, 22, and 56% when atypia and dysplasia were not regarded as malignant.

Follow-up data of at least 24 months after the initial bladder washing were obtained. Tumor development was evaluated in 68 patients with negative cystoscopic findings. Sixteen (18.6%) of these patients developed a tumor recurrence, of whom only one (1.5%) showed invasive disease. In Table 1, the prediction of tumor recurrence by cytologic means for the different pathologists is presented. For comparison, the ROC curves are given in Fig. 2. All expert scores resulted in AUCs lower than those of the BWK. In Fig. 3, the Kaplan-Meier curves are shown for the recurrence-free intervals for the different cytologists and the BWK. In the log-rank analysis, only the BWK showed a significant differ-

TABLE 2. Bladder Wash Cytology Findings of Expert Pathologists and Bladder Wash Karyometry in 18 Cases From Which Simultaneous Bladder Biopsy Specimens were available.

Sample	Bladder wash cytology				Bladder wash karyometry	Histologic Analysis	
	Pathologist A	Pathologist B	Pathologist C	Pathologist D		Stage	Grade
1	Negative	Negative	Negative	Negative	Intermediate	2	3
2	Atypia	Dysplasia or low-grade neoplasia	Negative	Negative	Low	a	1
3	Grade 1	Dysplasia or low-grade neoplasia	Unknown	Low grade	Intermediate	a	2
4	Negative	Negative	Negative	Low grade	Low	a	1
5	Grade 1	Negative	Negative	Negative	Low	a	2
6	Grade 3	High grade	Positive	High grade	High		cis
7	Negative	High grade	Suspicious	Negative	High	2	2
8	Grade 2	High grade	Positive	Low grade	High	2	2
9	Grade 1	High grade	Atypia	Negative	Intermediate	a	2
10	Grade 3	High grade	Positive	High grade	High		cis
11	Grade 2	High grade	Atypia	Negative	Low	a	1
12	Grade 2	High grade	Atypia	High grade	Intermediate	a	2
13	Negative	Negative	Unknown	Negative	High	1	2
14	Grade 3	High grade	Positive	High grade	High		cis
15	Grade 2	Dysplasia or high-grade neoplasia	Severe atypia	High grade	High		cis
16	Grade 2	Low grade	Suspicious	Low grade	Intermediate	a	2
17	Grade 2	Low grade	Suspicious	High grade	Low	a	2
18	Negative	Negative	Negative	Negative	High	a	2

cis, carcinoma *in situ*.



AUC (area under the ROC curve)			
BWC		AUC	s.e.
	a.	0.484	0.085
	b.	0.464	0.082
	c.	0.502	0.083
	d.	0.579	0.082
BWK (QUANTICYT)		0.689	0.081

FIGURE 2. ROC curves for the prediction of tumor recurrence after a normal cystoscopy (n = 68). The thin unmarked line (—) represents the AUC value of 0.5 that would be obtained when random sample assignment was applied.

ence (P = .0057) for tumor recurrence-free intervals between tumor and no-tumor samples.

To study the independent value of cytologic and image analysis, a multivariate analysis for both the prediction of a lesion at cystoscopic examination and tumor recurrence was performed. The addition of the BWK to the cytologic interpretation did not increase prediction of a bladder lesion at cystoscopic examination in logistic regression analysis. For the prediction of tumor recurrence, the BWK was the only valuable parameter in a multivariate Cox regression analysis with forward conditional entering (P to enter, .05; P to remove, .10; β = 1.2587; P = 0.01). When clinical parameters were entered in the Cox forward conditional regression analysis, the BWK in combination with the highest grade of the earlier resected tumor gave the best prediction of the chance of tumor recurrence, similar to the finding of an earlier investigation (10). We could not perform statistical analysis for the prediction of progression because of the low number of progressive tumors.

DISCUSSION

The measurement of any method for the detection of bladder cancer suffers from lack of a "gold" standard, *i.e.*, a test that would reliably reveal the true state of the bladder urothelium whenever the test was applied. In the absence of such a method, the reference point for any new technique must be somewhat arbitrary.

Image analysis systems have been used to quantify cellular and nuclear characteristics in bladder cancer cells (9, 10, 12–14). Koss *et al.* (13) introduced a system for the cell-to-cell analysis to assess

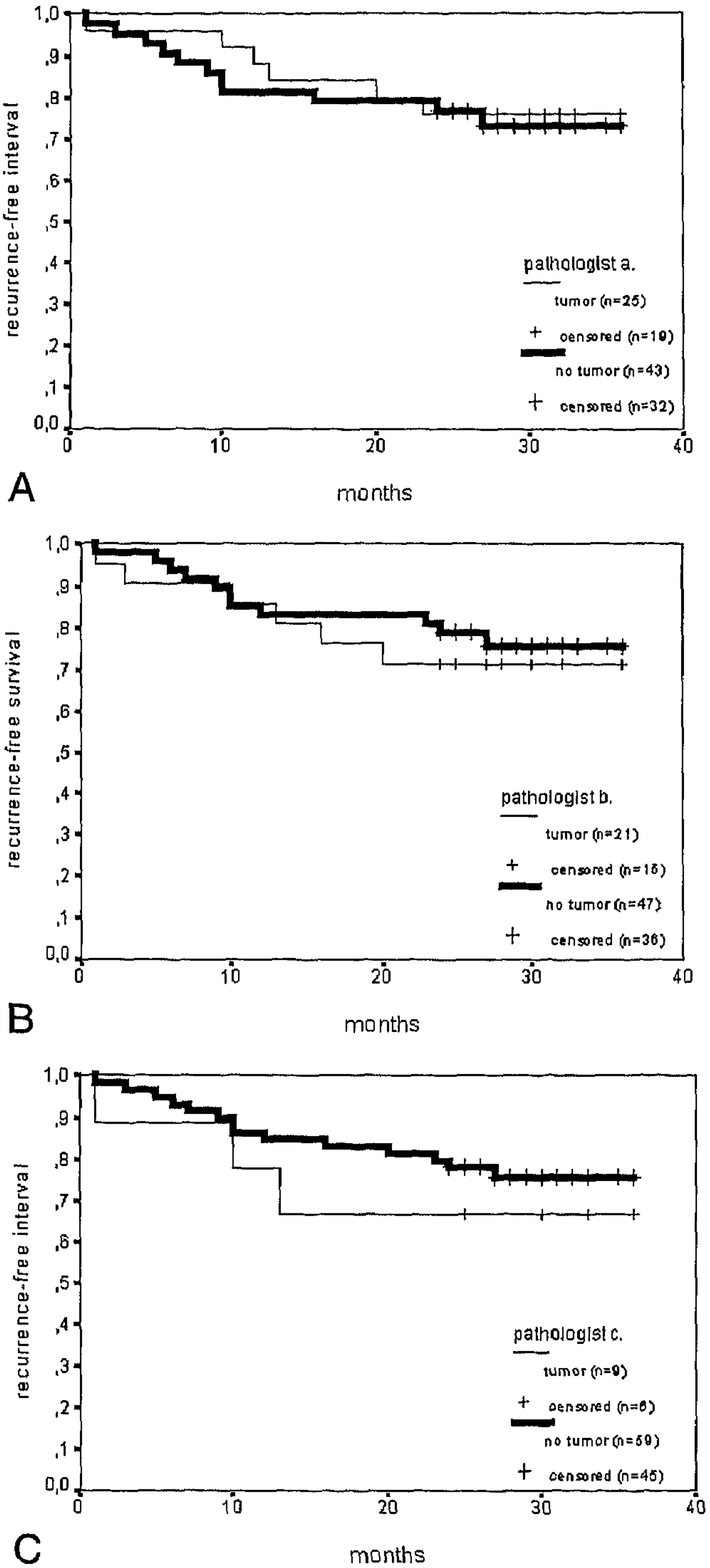


FIGURE 3. Kaplan-Meier presentations of recurrence-free intervals of the 68 patients with negative results from cystoscopic examination at the start of the study. Both BWC and BWK (QUANTICYT) are divided into tumor and no-tumor classes. The log-rank test was performed for comparison of the two groups.

the number of atypical cells per sample (13). The distribution of chromatin granules in the nucleus was found to be a powerful discriminator of normal and malignant cells in voided urine (13). Although initial results were promising, application of the system on a larger scale was never achieved. Later studies again confirmed the diagnostic value of chromatin texture image analysis (12, 15) in standardized populations. The disadvantage of applying textual features for diagnosis is that they are influenced by material processing (16).

DNA ploidy analysis is another method to aid in the interpretation of bladder cytology. Early flow cytometric studies showed clearly that aneuploidy was highly correlated with malignancy (3). Cajulis *et al.* (4) showed that image analysis DNA ploidy determination resulted in the highest sensitivity for tumor detection when compared to flow cytometry,

in situ hybridization methods, and cytology in bladder wash material. Unfortunately, low-grade tumor cells are often diploid and can not be discriminated from normal mucosal cells on the basis of ploidy analysis alone.

In this study, we chose to compare BWC and QUANTICYT to cystoscopy and (biopsy) histology. Murphy (1) extensively describes the criteria applied in urinary cytology. He concluded that experience is required for accurate grading. Hence, it is not surprising that low reproducibility was found when comparing different cytopathologists (17). The present study compared light microscopic cytologic examination to an image analysis method. Three aspects of the follow-up of bladder cancer patients were compared: cystoscopic findings, histologic features, and tumor recurrence.

Image analysis methods allow a more objective assessment of cellular and nuclear characteristics than does conventional light microscopic analysis, primarily because the criteria for analysis can be reduced in number, more clearly stated, and more consistently applied. An automated image analysis system (QUANTICYT) was developed and described in an analysis of 1412 patients (10). The QUANTICYT system applies nuclear DNA content (2c deviation index) (18) and nuclear shape to score bladder wash samples according to the risk of the presence or future appearance of bladder malignancies. The graphic representation of subsequent quantitative results facilitates interpretation of mucosal changes in the bladder (10). In earlier unpublished studies, the BWK was compared with cytologic interpretation. From these data, it seemed that cytologic analysis and BWK provided supplemental information. Recurrence and progression rates were highest in cases in which both methods agreed on the presence of malignant cells. In that study, the cytopathologist was aware of the result of the BWK.

Image analysis by QUANTICYT did not detect more cystoscopic lesions than did conventional light microscopic analysis by four experts in the field of cytology. Combining different scorings by either conventional cytologic or image analysis did not improve prediction of a cystoscopic lesion. The ROC curve analysis did not show significant differences among pathologists for the correlation with cystoscopic findings.

When the histologic diagnosis was compared with the BWC and the BWK results in 18 cases, it was found that all of the 4 CIS lesions were detected by both methods (Table 2). Of 10 low-stage, low-grade lesions, 8 were detected by (panel) cytologic analysis, whereas only 5 were found by BWK. In 2 of 18 cases, however, (panel) cytologic results were equivocal. In 3 (16.7%) of 18 cases, overall cytologic diagnosis was normal, whereas the BWK result showed intermediate or high risk, and a malignant lesion was found at histologic examination (in 2 cases, non-Ta). Conversely, the risk score was low in five cases, accompanied by an overall abnormal cytologic finding in four. All of the cases were pTa lesions. In one case, both the BWC and BWK were negative or only atypical, whereas a pTaG2 lesion was found at histologic examination. Follow-up in this case showed no recurrence after 34 months. From this small group of patients, it seems that expert (panel) cytologic assessment is more sensitive to low-grade, low-stage lesions than is BWK. In some cases, however, cytologic examination failed to detect lesions found by BWK that showed a more aggressive behavior at histologic analysis. Both methods failed to detect 1 (5.6%) of 18 tumors. It seems that both BWK and BWC are needed for the detection of histologically proven tumor.

The third comparison, the prediction of tumor recurrence after normal cystoscopic findings, was most reliably performed by BWK. Although tumor recurrences were seen after 7 of 50 low-risk BWK samples, the majority of tumor recurrences occurred in intermediate-risk and high-risk samples. Moreover, recurrence-free intervals for six of the seven recurrences after a low-risk BWK finding were longer than 12 months. An explanation for the superiority of BWK over BWC for the prediction of tumor recurrences could be the fact that the risk score is particularly sensitive to small changes in the DNA histogram, as quantified by the 2c deviation index. Subtle changes, *e.g.*, increased proliferation, will result in a 2c deviation index over 1.35 and thus in a high-risk BWK. Because these relatively small changes might not result in cytologically detectable abnormalities, they might be undetected visually. This also fits with the finding of the higher specificity in the cytologic grading than in the QUANTICYT risk score. From the comparison with histologic and follow-up data, it is apparent that BWK offers additional information to conventional cytologic assessment, even when the cytologic analysis is performed by experts.

CONCLUSION

Both expert BWC and BWK show comparable correlations with the presence of a cystoscopic lesion. Detection rates for histologically confirmed low-grade, low-stage lesions were 8 of 10 for cytologic analysis and 5 of 9 for BWK. Only 2 of 10 lesions, however, were detected by all of the 4 cytopathologists. All of the CISs were detected by both BWC and BWK. Prediction of tumor recurrence after normal findings at cystoscopic examination was best achieved by BWK. Image analysis can aid in a more complete diagnostic and prognostic assess-

ment when combined with light microscopic cytologic evaluation.

Acknowledgment: *The authors thank Dr. L.A. Kiemeney for advice on statistics and Mr. P. Peelen for technical support on the QUANTICYT analysis.*

REFERENCES

1. Murphy WM. Current status of urinary cytology in the evaluation of bladder neoplasms. Hum Pathol 1990;21:886–96.
2. Zein T, Waisman Z, Englander LS, Gamarra M, Lopez C, Huben RP, *et al.* Evaluation of bladder washings and urine cytology for the diagnosis of bladder cancer and its correlation with selected biopsies of the bladder mucosa. J Urol 1984;132:670–2.
3. Tribukait B, Gustafson H, Esposti PL. The significance of ploidy and proliferation in the clinical and biological evaluation of bladder tumors: a study of 100 untreated cases. J Urol 1982;54:130–3.
4. Cajulis RS, Haines GK, Frias-Hidvegi D, McVary K, Bacus JW. Cytology, flow cytometry, image analysis, and interphase cytogenetics by fluorescence *in situ* hybridization in the diagnosis of transitional cell carcinoma in bladder washings: a comparative study. Diagn Cytopathol 1995;13:214–23.
5. Amberson JB, Laino JP. Image cytometric deoxyribonucleic acid analysis of urine specimens as an adjunct to visual cytology in the detection of urothelial cell carcinoma. J Urol 1993;149:42–5.
6. Hemstreet GP, Rollins S, Jones P, Rao JY, Hurst RE, Bonner RB, *et al.* Identification of a high-risk subgroup of grade 1 transitional cell carcinoma using image analysis–based deoxyribonucleic acid ploidy analysis of tumor tissue. J Urol 1991;146:1525–9.
7. Murphy WM, Emerson LD, Chandler RW, Moinuddin SM, Soloway MS. Flow cytometry *versus* urinary cytology in the evaluation of patients with bladder cancer. J Urol 1986;136:815–8.
8. Norming U, Nyman CR, Tribukait B. Comparative flow cytometric deoxyribonucleic acid studies on exophytic tumor and random mucosal biopsies in untreated carcinoma of the bladder. J Urol 1989;142:1442–7.
9. van der Poel HG, Schaafsma HE, Vooijs GP, Debruyne FMJ, Schalken JA. Quantitative light microscopy in urologic oncology. J Urol 1992;148:1–13.
10. van der Poel HG, Witjes JA, van Stratum P, Boon ME, Debruyne FMJ, Schalken JA. QUANTICYT. Karyometric analysis of bladder washing for patients with superficial bladder cancer. Urology 1996;48:357–64.
11. van Velthoven R, Petein M, Oosterlinck WJ, Roels H, Pasteels JL, Schulman C, *et al.* The use of digital image analysis of chromatin texture in Feulgen-stained nuclei to predict recurrence of low-grade superficial transitional cell carcinoma of the bladder. Cancer 1995;75:560–8.
12. Harley JA, McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. Radiology 1982;143:29–39.
13. Koss LG, Bartels PH, Wied GL. Computer-based diagnostic analysis of cells in the urinary sediment. J Urol 1980;123:846–9.
14. Ooms EC, Kurver PJH, ME Boon. Morphometrical analysis of urothelial cells in voided urine of patients with low-grade and high-grade bladder tumours. J Clin Pathol 1982;35:1063–72.
15. Wiener H, van 't Hof-Grootenboer AE, Vooijs GP. Sum variance: a most valuable parameter in computer-assisted image analysis of voided urines. Cytopathology 1996;7:256–62.
16. Boon ME, van der Poel HG, Tan CJ, Kok LP. Effect of embedding methods *versus* fixative type on karyometric measures. Anal Quant Cytol Histol 1994;16:131–6.
17. Sherman AB, Koss LG, Adams SE. Interobserver and intraobserver differences in the diagnosis of urothelial cells. Comparison with classification by computer. Anal Quant Cytol Histol 1984;6:112–21.
18. Böcking A, Adler CP, Common HD, Hilgarth M, Granzen B, Auffermann W. Algorithm for a DNA cytophotometric diagnosis and grading of malignancy. Anal Quant Cytol 1984–6:1–11.